

susceptible to gentamicin, and 10 resistant. Results for chloramphenicol were: 22 strains susceptible, 3 intermediate, 2 resistant.

We have found 6 strains susceptible to erythromycin.

Neonatal infections

P312 Preterm labor and bacterial intra-amniotic infection: arachidonic acid liberation by phospholipase A₂ of *Fusobacterium nucleatum*

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Objectives: The studies presented in this report were undertaken to evaluate whether *Fusobacterium nucleatum*, a common anaerobic isolate of intrauterine infection, stimulates arachidonic acid metabolism, as a rate-limiting step for prostaglandin synthesis in the human uterine endometrium.

Study design: Effects of *F. nucleatum* on arachidonic acid liberation and *F. nucleatum* extract on lysophosphatidylcholine production from human uterine endometrial cells were investigated.

Results: When human uterine endometrial cells prelabelled with [³H]arachidonic acid to an isotopically steady state were exposed to an extract of *F. nucleatum*, arachidonic acid liberation was stimulated, accompanied by lysophospholipid formation. A similar stimulatory effect on phospholipid degradation was observed in the experiment with the bacterial conditioned media.

Conclusions: These results suggest that *F. nucleatum* stimulates endometrial phospholipid metabolism, related to the activity of phospholipase A₂, which might induce the onset of labor associated with intra-amniotic infection.

P313 Congenital toxoplasmosis: evaluation of infection risk in the newborn on the basis of the maternal immunologic situation

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Objectives: In a period of 12 years, 162 newborns at risk of congenital toxoplasmosis were identified. The criteria of the European Research Network on Congenital Toxoplasmosis were used in order to obtain a more correct framing of risk of transmission.

Methods: Of the 162 children, 46 (29%) were born to mothers with certain infection, 36 (22.4%) to mothers with probable infection, 43 (27%) to mothers with possible infection and 34 (21.4%) to mothers with doubtful infection (159 pregnancies). The transmission of the infection to offspring occurred in 13 cases (8.2%): 11 cases out of 46 (23.9%) in certain infections, 2 cases out of 36 (5.5%) in probable infections and none in possible and in doubtful infections. Only the 82 children of mothers with certain (46 cases) or probable (36 cases) infection were considered.

Results: Infection occurred in 13 cases out of 82 (15.8%). Infection was never transmitted in the first trimester but in 6 cases out of 27 (22.2%) in the second and in 7 cases out of 17 (41.1%) in the third trimester. Infection was symptomatic in two children (15.3%) of infected newborns and 2.4% of 82 newborns at risk, both from mothers with certain and probable infection in the second trimester of pregnancy.

Conclusions: (1) Groups of newborns at higher risk of infection have been identified: these children should receive long-term follow-up; (2) diagnosis, follow-up and therapy protocols must be formulated on the basis of the risk.

P314 Toxo-net: a survey of congenital toxoplasmosis in the region of Piedmont (Italy)

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Objectives: Toxo-net is trying to improve the management quality of laboratory tests and results by means of 'directing-lines'.

Methods: in December 1996 a survey network for congenital toxoplasmosis was set up, which involves pathologists, obstetricians and neonatologists from 30 hospitals in Piedmont (31 neonatal, 32 obstetric and 29 laboratory units).

The aims are: to estimate the incidence of the problem in our regions, to verify the reliability and effectiveness of case definition, and to identify and discuss diagnostic, therapeutic and follow-up protocols for pregnant women and newborns. The participants were given the classification and case definitions system (as stated by the European Network on Congenital Toxoplasmosis) of infection in pregnancy and in newborns, and the protocols of prenatal diagnosis, of therapy in pregnant women and newborns and of neonatal follow-up. Each unit communicates every fourth month the cases being observed and followed.

Results: At the end of 1997, 19 neonatal units (21 500 out of 33 000 newborns/year) and 22 laboratories (28 000 pregnant women/year) had performed an active survey with documented cases.

Conclusions: The different laboratories still do not have a homogeneous way of choosing first- and second-level tests. Furthermore, the criteria for the final diagnostic evaluation are often not comparable.

P315 Colonization/infection caused by *Klebsiella pneumoniae* (Kp) isolates producing extended-spectrum beta-lactamases (ESBLs) in a neonatal unit (NU)

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Objectives: To survey the extent of colonization/infection due to Kp ESBL among neonates admitted to our unit. This was prompted by the admission of 6 neonates with urinary tract infection caused by Kp ESBL, referred to us from the same maternity hospital (MH) in a short period of time (26 May 1998 to 27 August 1998).

Methods: Medical records of infants admitted to our NU between 1 January 1997 and 31 October 1998 were reviewed. Also, two 1-day epidemiologic surveys were carried out which involved the collection of throat and rectal swabs from the babies on the NU and of samples from the medical and nursing staff, instruments and water taps. Antimicrobial susceptibility was tested by the disk diffusion method and detection of ESBL isolates was performed by double disk synergy test (DDST).

Results: The NU located in a children's hospital accepts neonates for tertiary care. During the study period, 903 neonates were admit-

ted. Fifty-four neonates were colonized/infected by Kp ESPL. Of these, 37 were already colonized/infected on admission (30/37 coming from two MHs in the Athens region) and 17 were colonized/infected on our NU. The infected neonates were initially treated with gentamicin and later this was modified according to the in vitro susceptibility, if necessary. All survived. The source of Kp ESBL cross-infection was found to be the weighing scales and appropriate measures were taken. Relevant MHs were notified of the problem and advice was given.

Conclusions: Vigilant infection control measures at referring MHs should be enforced, as neonates colonized with ESBL strains can be a significant environmental hazard and a therapeutic problem.

P316 Bacterial colonization of the newborns

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Objectives: To study the colonization with Gram-negative bacteria in 730 infants admitted in a 30-bed neonatal ward during a 2-year period (1 October 1995 to 30 September 1997).

Methods: Surface cultures were taken from neonates upon admission and then once weekly until discharge. Cultures were performed with conventional methods and the susceptibility testing with a standard disk diffusion method.

Results: Fifty-three per cent of the infants were found to be colonized with 760 Gram-negative strains. The total colonization rates of the upper respiratory tract, the rectum and the umbilicus were 35.8%, 57.3% and 30.1% respectively. The table shows the predominant organisms and their resistance to antimicrobials:

	<i>E. coli</i> n=269	<i>Klebsiella</i> n=156	<i>Enterobacter</i> n=107	<i>Serratia</i> n=37	<i>Acinetobacter</i> n=26	<i>P. aeruginosa</i> n=67
Ampicillin	58%	100%	100%	100%	76.9%	-
Amox.+Clav.	5.6%	9%	88.8%	100%	19.2%	-
Cefotaxime	1.1%	14.1%	25.2%	83.8%	69.2%	85.1%
Ceftazidime	1.1%	21.8%	31.8%	81.1%	69.2%	28.4%
Imipenem	0%	0%	0.9%	0%	3.8%	1.5%
Gentamicin	1.5%	13.5%	20.6%	62.2%	19.2%	11.9%
Amikacin	0.7%	16.7%	20.6%	70.3%	19.2%	6.0%
Cotrimoxazole	9.3%	16.7%	26.2%	78.4%	19.2%	-

Conclusions: The high colonization frequency of neonates by multiresistant Gram-negative organisms, other than *E. coli*, must be taken into account in the decisions regarding the appropriate empirical regimens for the treatment of related infections.

P317 Evidence of nosocomial cross-infection by *Stenotrophomonas maltophilia* in a neonatology unit

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Objectives: To characterize by molecular methods an outbreak of *S. maltophilia* in the neonatology unit of our hospital.

Methods: 11 *S. maltophilia* isolates obtained from 7 neonates within a 4-month period were typed by three different molecular methods: AP (arbitrarily primed) PCR, PFGE (pulsed-field gel electrophoresis) and ERIC (enterobacterial repetitive intergenic consensus) PCR. As unrelated controls, one strain isolated from another ward within the same period and another one from the same neonatology unit but isolated in a different period were also included. All fingerprint data were processed to obtain a similarity dendrogram.

Results: All related isolates except one showed a remarkably high homology among their fingerprints for the three methods assayed. They clustered at 96% similarity in the genetic relatedness dendrogram obtained from the typing profiles. Clearly different fingerprints were obtained for the unrelated isolates and they were unclustered in the dendrogram. The index case was considered to be a newborn who had an *S. maltophilia* isolate from a culture drawn on admission to the neonatology unit.

Conclusions: The high genetic similarity found for all but one of the related isolates, together with the presence of an index case, constitute the first evidence for the role of cross-infection in the nosocomial transmission of *S. maltophilia*.

P318 Toxin production in *Staphylococcus aureus* isolated from SIDS

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Objectives: Staphylococcal enterotoxin A (SEA), B (SEB), C (SEC) and D (SED) and toxic shock syndrome toxin-1 (TSST-1) production was measured in 12 *Staphylococcus aureus*, three *Staphylococcus haemolyticus* and two *Staphylococcus epidermidis* strains isolated from nasopharyngeal swabs, lung tissues and heart blood of seven infants who died suddenly.

Methods: Production of enterotoxins A, B, C and D, as well as TSST-1, was detected using Oxoid Toxin Detection Kit TD 940 and TD 900 (Oxoid Unipath Ltd, Hampshire, UK) according to the prescription of the manufacturer. Titers of toxins were measured by a serial double dilution of the supernatant of 24-h cultures.

Results: All but one *S. aureus* strains were toxin producers (92%), while none of the *S. haemolyticus* and *S. epidermidis* strains excreted any toxin. Among the 11 SEA-producing *S. aureus* strains (92%), eight (73%) had high titer ($\geq 1:8$ dilution) of toxin. Four of 11 strains produced SEB (36%), two in high titer. Nine (75%) proved to be TSST-1 producers. The simultaneous production of SEA, SEB and TSST-1 was exhibited by five strains (42%), that of SEA and TSST-1 by four strains (33%), and two strains produced SEA alone (17%), while one (8%) was lacking in toxin production. None of the strains elaborated staphylococcal enterotoxins C and D.

Conclusions: The high prevalence of toxin production and co-production in *S. aureus* strains from sudden infant death syndrome (SIDS) strongly support our supposition that staphylococcal superantigen effects may represent one of the multifactorial pathomechanisms of SIDS.

P319 Neonatal septicemia: a 7-year retrospective survey

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Objectives: To determine the epidemiology, microbiology and antibiotic susceptibility of neonatal septicemia in a busy tertiary center.

Methods: During the 7-year period (1991-97), 217 cases of blood culture-proven neonatal sepsis (179 inborns and 38 outborns) were evaluated retrospectively.

Results: The incidence rate of septicemia among the inborn newborns was 5.7 per 1000 live births. 47% of the total cases were premature. Early-onset sepsis was suffered by 48% of the septic newborns. Maternal risk factors were identified in 28.5% cases of

early-onset sepsis and in 6.6% of late ones. The predominant bacterial pathogens were: *Staphylococcus epidermidis* 35.9% (78 neonates), *Staphylococcus aureus* 19.4% (42), *Serratia* spp. 11% (24), *E. coli* 9.2% (20), *Enterococcus faecalis* (group D) 5.1% (11), *Streptococcus* spp. 2.6% (8), *Enterobacter cloacae* 2.3% (5), *Candida* spp. 2.3% (5), and *Hafnia alvei* 1.4% (3). The overall case-fatality rate was 11.5%. *Enterobacter cloacae* and *Candida* spp. had the highest mortality (80% 4/5 and 70% 3/5 respectively). The resistance rate of the microorganisms to the antibiotics used was as follows. *Staphylococcus epidermidis*: ampicillin (AM) 69.8%, gentamicin (GM) 60.9%, ceftazidime (CAZ) 28.9%, amikacin (AN) 8.2%, cefotaxime (CTX) 22.7%. *Staphylococcus aureus*: AM 50.7%, GM 33.8%, CAZ 8.3%, AN all sensitive, CTX 10.1%. *E. coli*: AM 30.8%, GM, CAZ, AN, CTX, all sensitive. *Serratia marcescens*: AM 46%, GM, CAZ, AN, all sensitive, CTX 22.7%. Ampicillin and gentamicin had the highest resistance rates among the antibiotics used.

Conclusions: Neonatal septicemia is still a major problem in NICU. The high resistance rate of the first-line antibiotics leads to therapeutic dilemmas.

P320 Cost of nosocomial infection in a neonatal intensive care unit

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Objectives: To determine the influence of nosocomial infection (NI) on the duration of hospital stay and cost.

Patients: All patients admitted to the Neonatal Intensive Care Unit of University Hospital Antwerp and who survived, between 1 November 1993 and 1 December 1995.

Methods: Factors associated with duration of hospital stay were used as matching

criteria. Cases of NI were defined as neonates with clinical or laboratory signs of infection for which antimicrobial therapy was started after 48 h of admission. Controls were matched to cases for gestational age, ventilator requirement, surgery and patent ductus Botalli. Nosocomial sepsis (NS) was defined as a patient with NI with a positive blood culture.

Results: *Patients:* Sixty-nine out of 515 (12.5%) neonates developed NI. 45 cases were matched. From these, 20 (40%) had a proven NS. *Hospitalization duration:* The mean duration of hospitalization was 30 days for controls and 54 days for neonates with NI ($p < 0.002$). Patients with proven NS had the same duration compared to those without proven NS: 52 versus 67 days ($P = 0.05$). *Costs:* The total extra cost due to NI was 11 030 ECU (NI = 23,193 ECU, controls = ECU 12,172, $P < 0.001$). The extra cost is due to charges for hospitalization (65%), pharmaceutical cost (11%) and honoraria (23%). Patients with NS and those without proven NS had the same total cost as well as pharmaceutical costs.

Conclusions: The extra duration of hospitalization due to NI in neonates is responsible for a major extra cost. Prevention of NI and more accurate diagnosis of NS might decrease the total cost of care.

P321 Variation of bloodstream isolate distribution in a neonatal intensive care unit from 1993 to 1997

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Objectives: To determine the isolation rate (IR) of bloodstream isolates and its evolution in an NICU during a 5-year period.

Methods: In a retrospective study we analyzed the microbiological data of blood cultures (BC) performed from 1993 to 1997 of neonatal patients (pts) hospitalized in a 30-bed ICU.

Results: In the considered period we performed 2829 BC of 951 pts, with a mean of BC/pt increase from 2 in 1993 to 4.3 in 1997. 274 pts had positive BC (28.8%); of those, 107 had ≥ 2 positive BC. The total of isolates was 604. The microorganism most frequently isolated was *S. epidermidis* (SE), 288/604 strains (IR 47.6%), with a statistically significant increase ($p < 0.01$) of pts with 1 pos BC (49/351 in 1993–94 and 102/419 in 1996–97) and ≥ 2 positive BC (8/351 in 1993–94 and 43/419 in 1996–97). The IR of other coagulase-negative staphylococci was 15.4%, with *S. hominis* and *S. warneri* recovered from ≥ 2 BC in 5/951 pts. *S. aureus* (IR 10.7%) was isolated in 40 pts (17 with ≥ 2 positive BC) while *Candida* spp. were isolated (IR 7.8%) in 14 pts (12 with ≥ 2 positive BC) and Gram negatives (IR 7.6%) were isolated in 31 pts.

Conclusions: Our data show: (1) SE is now the most important pathogen in NICUs, also regarding its antibiotic resistance, (2) isolation of *Candida* spp. from BC is always significant, (3) the increase in the number of BC/pt improved the microbiological diagnosis of possible bloodstream infection caused by nosocomial pathogens.

P322 The fecal colonization and the epidemiology of a TEM-10-producing *Klebsiella pneumoniae* strain in a Portuguese neonatal ward

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Klebsiellae are opportunistic pathogens and can give rise to several and severe infections. Typically, *klebsiella* infections are associated with hospitalization. Apart from medical equipment and blood products, the principal reservoirs for transmission of *Klebsiella* are the gastrointestinal tract of patients and hands of hospital personnel. In pediatric wards, nosocomial *klebsiella* infections are especially troublesome, and an increasing number of endemic and epidemic outbreaks has been reported. At the Neonatal Intensive Care Unit of Hospital Santa Maria Lisboa, a TEM-10-producing *Klebsiella pneumoniae* strain, that was an endemic strain in this hospital since 1991, was isolated from a blood culture, in one of the neonates.

To evaluate the carriage rate, rectal swab specimens were taken from a total of 20 neonates and were investigated with respect to the isolation of a TEM-10-producing *Klebsiella pneumoniae* strain.

Fifty-five per cent of neonates studied were colonized with ceftazidime-resistant *Klebsiella* strains. Although bacterial strains share an extended-spectrum beta-lactamase, identified as a TEM-10, genomic analysis by PFGE (pulsed-field gel electrophoresis) revealed two different electrophoretic profiles that were different from those of all other isolates studied from the other wards in this hospital.

P323 Etiologic structure of nosocomial infection among newborns

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Objectives: To determine the etiologic structure of nosocomial infection among newborns at NICUs in Moscow, Russia.

Methods: Blood culture tests were performed among 258 newborns at NICU suspicious on presence of sepsis. Blood samples were taken from babies who required antibacterial therapy in the first and second weeks of life. Between one and three specimens were taken from each newborn. All blood cultures were isolated by the routine method. Identification was performed with BBL Crystal ID and Minitek (Becton Dickinson), as recommended by the manufacturer.

Results: 667 blood samples were analyzed; in 456 cases, neither bacteria nor fungi were detected, and in 210 cases blood culture was positive. Organisms found were coagulase-negative staphylococci (73%), *Staphylococcus aureus* (5%), *Streptococcus faecium* (5%), *Klebsiella pneumoniae* (3%), *Pseudomonas aeruginosa* (4%), *Acinetobacter* spp. (2%) and *Candida* spp. (8%). The prevalent role belonged to Gram-positive microorganisms (83%); among them methicillin-resistant *Staphylococcus aureus* (MRSA) and *Enterococcus* spp. became the most problematic agents, causing great difficulties during therapy. About 70% of isolated *Staphylococcus aureus* strains had resistance for methicillin.

Conclusion: glycopeptides are drugs of choice for MRSA and *Enterococcus* spp. related sepsis in neonatal period at NICU.

P324 Candidemia in low-birthweight premature neonates treated with fluconazole. Report of 26 cases, seven of them complicated with fungal meningitis

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Objectives: Twenty-six low birth weight (750–1600) premature neonates (30.5 median gestation week) with *C. albicans* fungaemia were treated with intravenous fluconazol in daily dose 6 mg/kg once daily for 6–48 days.

Methods: Twenty-six low-birthweight (750–1600) premature neonates (30.5 median gestation week) with *C. albicans* fungemia were treated with intravenous fluconazole at a daily dose of 6 mg/kg once daily for 6–48 days.

Results: Twenty-three (88.5%) were cured; however, three of them relapsed despite at least 14 days of therapy but were ultimately cured without sequelae. Three other neonates died, two of them due to fungal infection and one because of prematurity and lung failure with fungemia (11.5% overall and 7.9% attributable mortality). Two neonates developed elevated liver enzymes and two other elevated serum creatinine during therapy; however, in none of them did this lead to the discontinuation of therapy. In seven neonates, fungal meningitis developed as a complication of fungemia.

Conclusions: In conclusion, fluconazole seems to constitute safe and effective antifungal therapy in complicated or uncomplicated fungemia due to *C. albicans*.

Parasitic diseases

P325 Comparison of enzyme immunofluorescence assay (ELFA, VIDAS System) with the indirect immunofluorescence test (IIF) in serologic diagnosis of toxoplasmosis

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Toxoplasmosis is a widely spread parasitosis, and the determination of appearance and persistence of infection has great clinical importance in immunocompromised patients and in pregnant women.

From February 1997 to February 1998 in our laboratory, 828 sera were examined for the presence of specific IgM and IgG antibodies against toxoplasmas. Two commercial tests were used; automated enzyme fluorescent immunoassay for the detection of specific IgM and IgG, and indirect immunofluorescence test (both tests, bioMérieux, France). Of the total number of sera, in 357 specific IgG were detected in both tests, while in 5 high IgG titers were found. Of the total sera tested by ELFA, in 17 specific IgM antibodies were found, in 15 with the IIF test, and all of them proved to be from the group positive for the IgG-specific antibodies. By the IIF method, IgM antibodies were confirmed in 88% of the total sera confirmed by the ELFA method. Using the correlation test (Pearson product-moment correlation), significant correlation was found between the IgM and IgG values measured by ELFA and IIF tests (IgM $r=0.73$, $p=0.001$; IgG $r=0.70$, $p=0.001$). In the t-test for independent samples, significant differences between methods were not found ($p=0.05$). We did not find significant differences between the two methods in which high diagnostic sensitivity and specificity were verified in few comparative studies. We consider that ELFA and IIF are reliable tests for the detection of toxoplasma-specific IgM and IgG antibodies present in serum.

One part of this work was presented at 1. Croatian Congress in Infectious Diseases.

P326 Toxoplasmosis antibody prevalence in pregnancy in Buenos Aires Province, Argentina

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Objectives: To study IgG and IgM antibody prevalence in pregnant women at risk of infection with *Toxoplasma gondii*.

Materials and methods: 1300 blood samples of 6–50 pregnant women were tested during 1 year. Samples were extracted with 3 weeks' difference. All of them were tested for IgG antibodies by MEIA (Toxo 2.0 Abbott). IgM specificity was tested by ISAGA (Toxo-ISAGA Biomerieux) in the following cases only: (A) IgG quantification over 300 UI/mL; (B) seroconverted; (C) the titer was so different that it suggested acute infection.

Results: IgG antibodies were detected in 347 (53.4%) out of the 650 patients studied. IgM antibodies were tested in 15 (2.31%) of these patients with the following results. Eight patients showed IgG antibody titers over 300 UI/mL. On testing IgM, 6 of them showed I.I.=12 (2 were also HIV co-infected). The other 2 were negative. Seroconverted patients showed I.I.=10. Of the 6 patients with a high IgG titer difference, 5 were negative and 1 showed I.I.=12.

Conclusions: (1) Toxoplasmosis IgG prevalence was 53.4%, with 1.23% acute infection (8 of them IgM positive). (2) Laboratory investigation detected 8 IgM-positive antibody patients with no clinical